Listing of Claims:

1. (Currently amended) An in vitro method of activating protein kinase B comprising the steps of:

(a) washing insulin responsive cells in ice-cold buffer and maintaining said cells at $4^{o}C$,

(b) incubating said insulin responsive cells from (a) in the presence of insulin,

(c) obtaining from said insulin-responsive cells from (b) a membrane fraction ~~fraction,~~ which comprises (i) an insulin receptor, (ii) an IRS-1("insulin receptor substrate -1"), (iii) an IRS-2 ("insulin receptor substrate -2"), (iv) a p85 subunit of PI-3 Kinase ("phosphatidyl-inositol 3-kinase") and (iv) PDK2 ("phosphoinositide-dependent kinase 2") activity, and a cytoplasmic fraction ~~fraction,~~ which comprises (i) PDK1 ("phosphoinositide-dependent kinase 1") activity, (ii) an IRS-1("insulin receptor substrate -1"), (iii) an IRS-2 ("insulin receptor substrate -2"), (iv) a p85 subunit of PI-3 Kinase ("phosphatidyl-inositol 3-kinase") and ~~which comprises~~ (v) a protein kinase B, wherein said protein kinase B is not activated,

~~(b)~~ (d) preparing an assay mixture comprising the membrane fraction, the cytoplasmic fraction and ATP in a buffer comprising less than 145 mM chloride,

~~(c) optionally adding a phosphatidylinositol phosphate compound to the assay mixture,~~ and

~~(d)~~ (e) incubating said assay mixture at about $37^{o}C$ for up to about 15 minutes;

~~Wherein~~ wherein following step (e) said insulin receptor is autophosphorylated, said IRS-1 and said IRS-2 are phosphorylated, said p85 subunit of PI 3-kinase is bound to said IRS-1 and to said IRS-2, a PI 3-kinase is activated, said PDK1 is activated, said PDK2 is activated, and said protein kinase B is activated in the assay mixture by virtue of having a threonine residue phosphorylated and a serine residue phosphorylated such that the activated protein kinase B is capable of phosphorylating a GSK3 ("glucose synthase kinase-3"), thereby activating protein kinase B in vitro.

2. (Cancelled)

3. (Currently amended) The method of Claim 1 ~~claim 2~~ wherein the membrane fraction is a plasma membrane fraction.

4. (Original) The method of Claim 1 wherein the serine residue is at a position corresponding to amino acid 473 of SEQ ID NO:1 and the threonine residue is at a position corresponding to amino acid 308 of SEQ ID NO:1.

5. (Previously presented) The method of Claim 1 further comprising the step of combining PIP3 ("phosphatidylinositol 3,4,5-triphosphate") or PI(3,4)P2 ("phosphatidylinositol 3,4-biphosphate") with the membrane fraction, the cytoplasmic fraction and ATP in a buffer comprising less than 145 mM chloride.

6. (Original) The method of Claim 5 further comprising the step of combining PIP3 with the membrane fraction, the cytoplasmic fraction and ATP in a buffer comprising less than 145 mM chloride.

7. (Original) The method of Claim 1 wherein the insulin-responsive cell is a muscle cell, a liver cell, an adipocyte or an islet cell.

8. (Original) The method of Claim 1 wherein the insulin-responsive cell is an adipocyte.

9. (Currently amended) An in vitro method of activating protein kinase B comprising:

(a) obtaining from an insulin-responsive cell a plasma membrane fraction ~~fraction,~~ which comprises a PDK2 ("phosphoinositide-dependent kinase 2") activity and a cytoplasmic fraction ~~fraction,~~ which comprises a protein kinase B and a PDK1 ("phosphoinositide-dependent kinase 1") activity,

(b) treating said plasma membrane fraction with a solution comprising at least 145 mM chloride, thereby obtaining a salt-extracted plasma membrane fraction and an aqueous fraction,

(c) desalting the aqueous fraction thereby producing a desalted aqueous fraction comprising less than 145 mM chloride and said PDK2 ("phosphoinositide-dependent kinase 2") activity,

(d) preparing an assay mixture comprising the salt-extracted plasma membrane fraction, the cytoplasmic fraction, the desalted aqueous fraction, ATP, and a phosphatidylinositol phosphate molecule in a buffer comprising less than 145 mM chloride, wherein

(e) the protein kinase B is activated in the assay mixture by virtue of having a threonine residue phosphorylated and a serine residue phosphorylated, such that

(d) the activated protein kinase B is capable of phosphorylating a GSK3.

10. (Original) The method of Claim 9 wherein the serine residue is at a position corresponding to amino acid 473 of SEQ ID NO:1 and the threonine residue is at a position corresponding to amino acid 308 of SEQ ID NO:1.

11. (Original) The method of Claim 9 wherein the insulin-responsive cell is a muscle cell, a liver cell, an adipocyte or an islet cell.

12. (Original) The method of Claim 9 wherein the insulin-responsive cell is an adipocyte.

13. (Original) The method of Claim 9 wherein the insulin-responsive cell is treated with insulin.

14. (Original) The method of Claim 9 wherein the phosphatidylinositol phosphate molecule is a PIP3 or PI(3,4)P2.

15. (Original) The method of Claim 9 wherein the phosphatidylinositol phosphate molecule is a PIP3.

16-29. (Cancelled)

30. (New) The method of Claim 1 wherein said insulin responsive cells are maintained at 4°C in step (b).

31. (New) The method of Claim 1 further comprising the step of adding a phosphatidylinositol phosphate compound to the assay mixture in step (d).

32. (New) The method of Claim 1, wherein said membrane fraction and said cytoplasmic fraction are each maintained at 4°C in step (c).